(12) UK Patent Application (19) GB (11) 2 318 732 (13) A

(43) Date of A Publication 06.05.1998

(21) Application No 9622805.1

(22) Date of Filing 01.11.1996

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(51) INT CL[6]
**A61K 38/57 38/55 // C07K 14/81**

(52) UK CL (Edition P )
**A5B BHA B170 B31X B31Y B826**
**U1S S2416**

(56) Documents Cited
**EP 0648838 A1**
**J. Invest. Dermatol., (1995), 105 (4), pages 572-578**

(58) Field of Search
UK CL (Edition O ) **A5B BHA BJB**
INT CL[6] **A61K 38/55 38/57 , C07K 14/81**
**ONLINE: CAS-ONLINE; PHARM; WPI**

# (54) Wound healing compositions containing alpha-1-antitrypsin

(57) The invention provides the use of alpha-1-antitrypsin for the preparation of a composition for the treatment of a chronic wound, such as a pressure sore or a venous ulcer. The composition is preferably a wound dressing composition, such as an ointment for topical application.

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**FIG. 1**

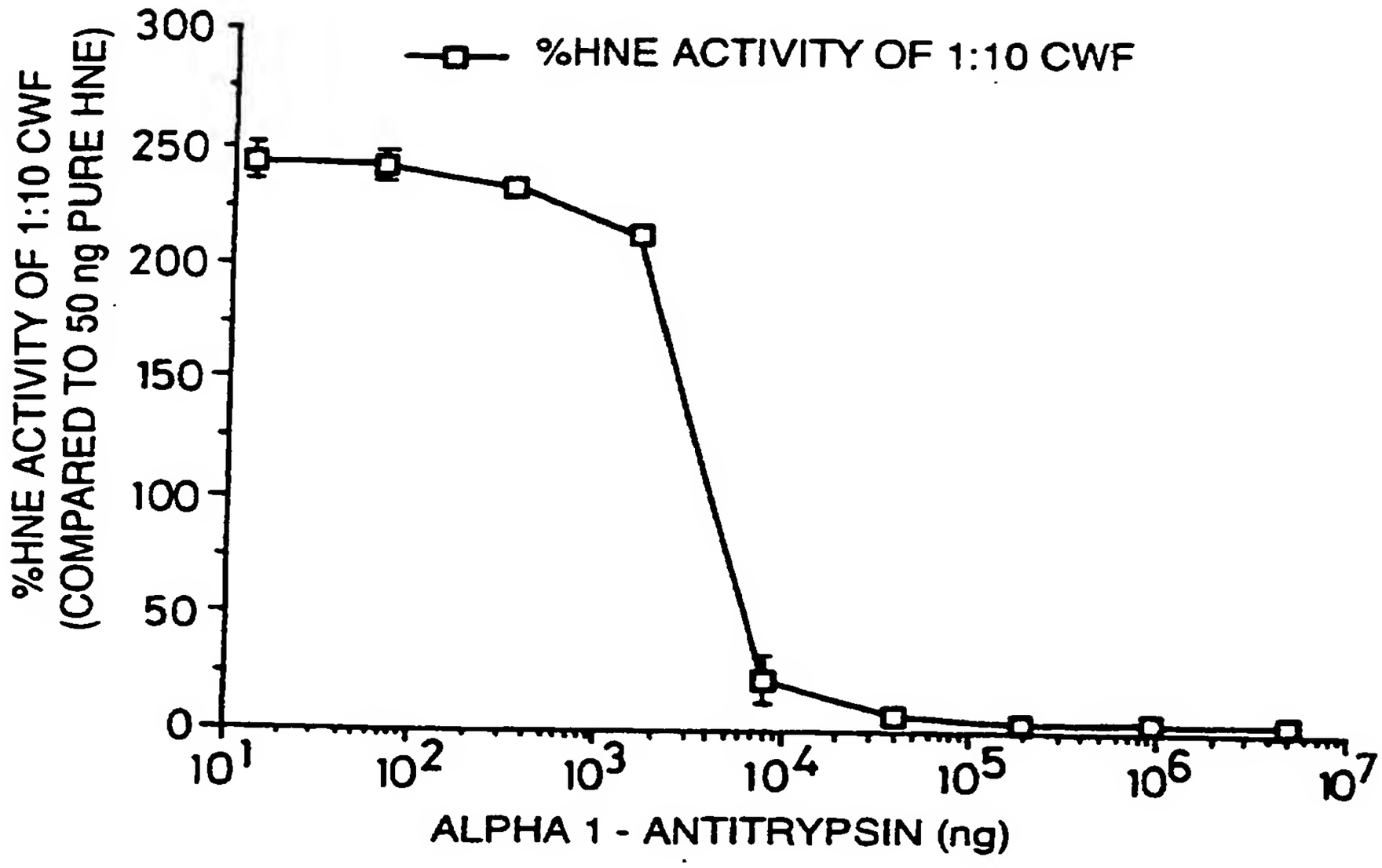

%HNE ACTIVITY OF 1:10 CWF
%HNE ACTIVITY OF 1:10 CWF (COMPARED TO 50 ng PURE HNE)
300
250
200
150
100
50
0
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^7$
ALPHA 1 - ANTITRYPSIN (ng)

**FIG. 2**

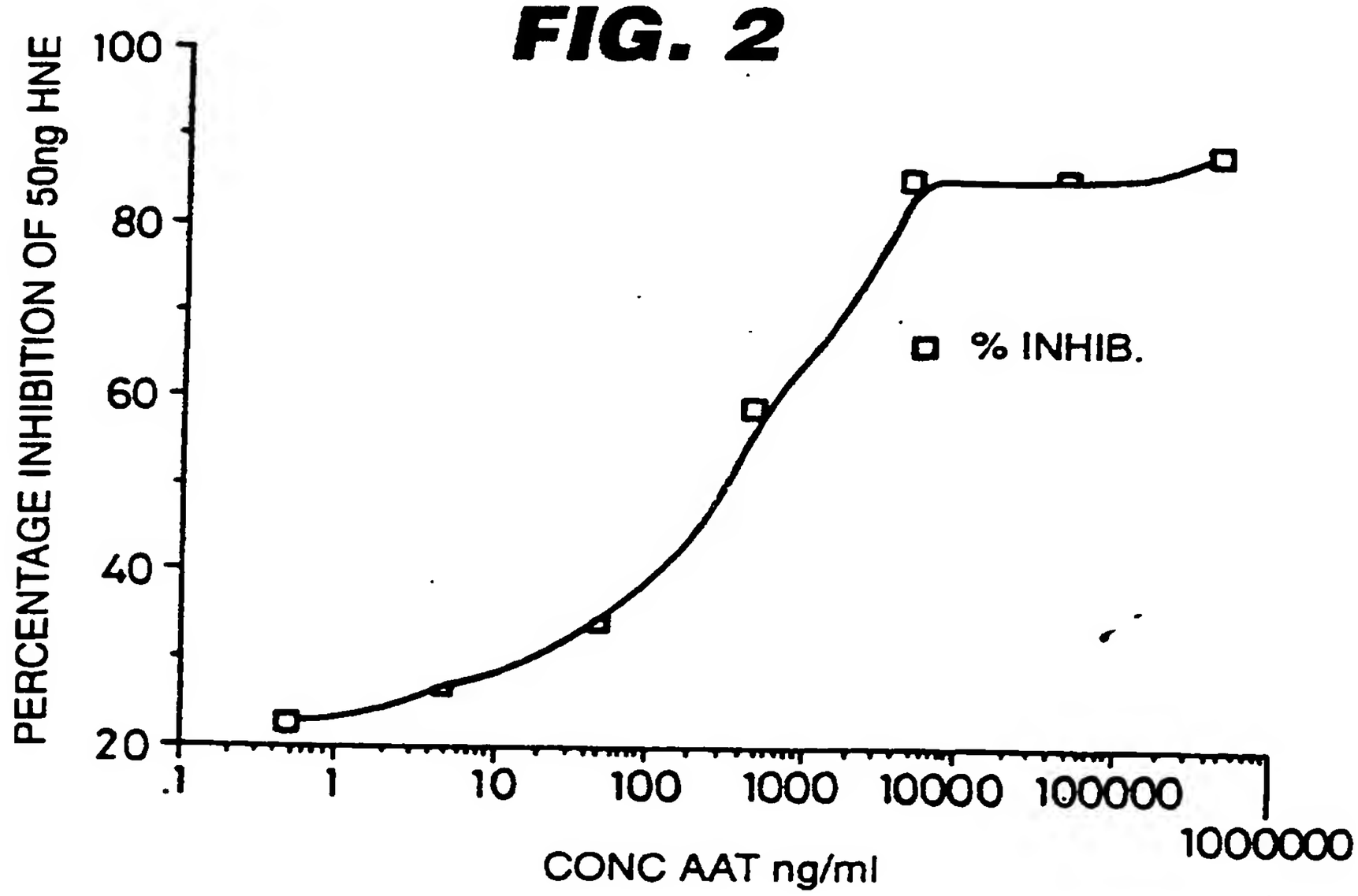

PERCENTAGE INHIBITION OF 50ng HNE
100
80
60
40
20
.1
1
10
100
1000
10000
100000
1000000
% INHIB.
CONC AAT ng/ml

**FIG. 3**

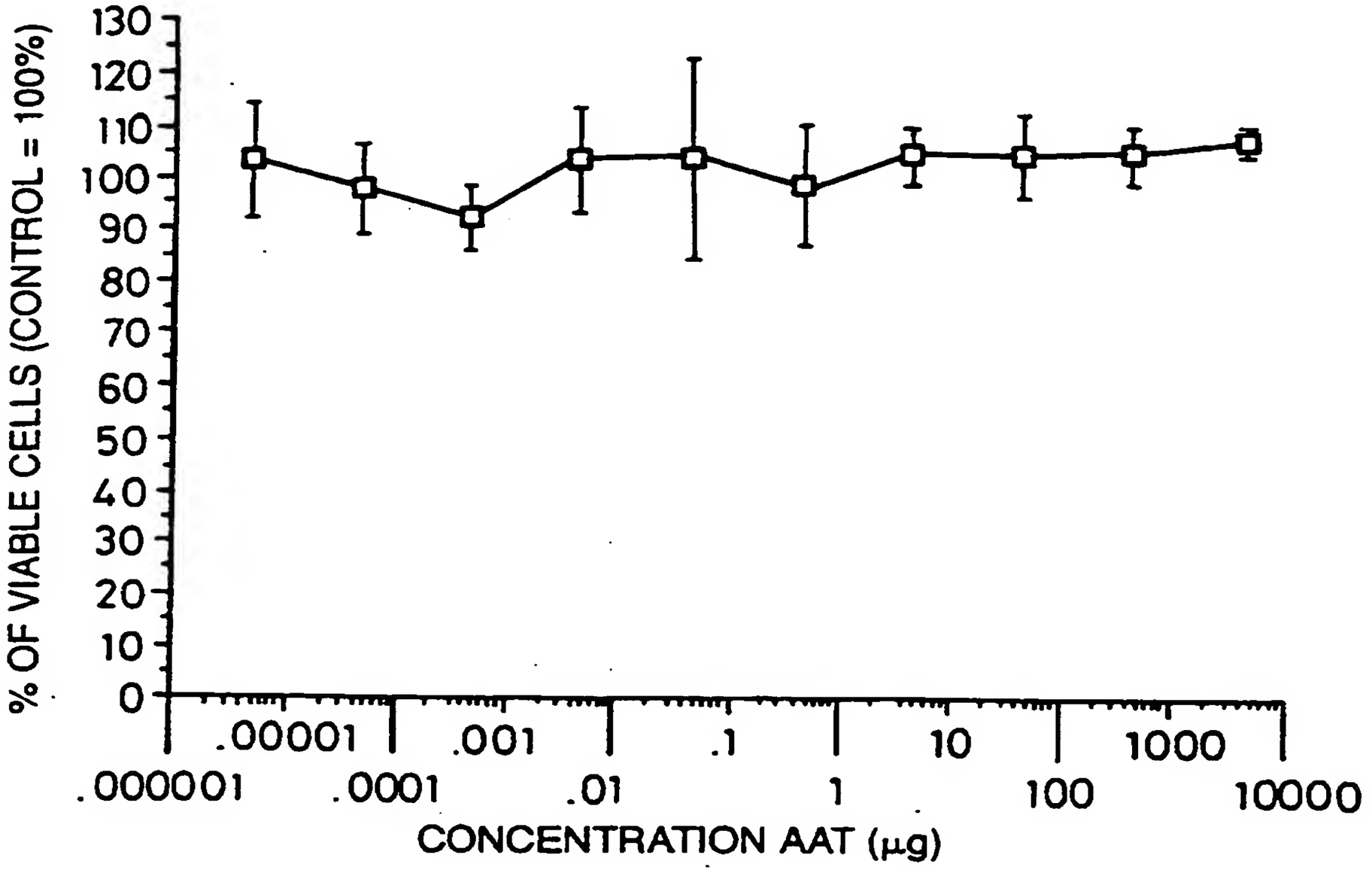
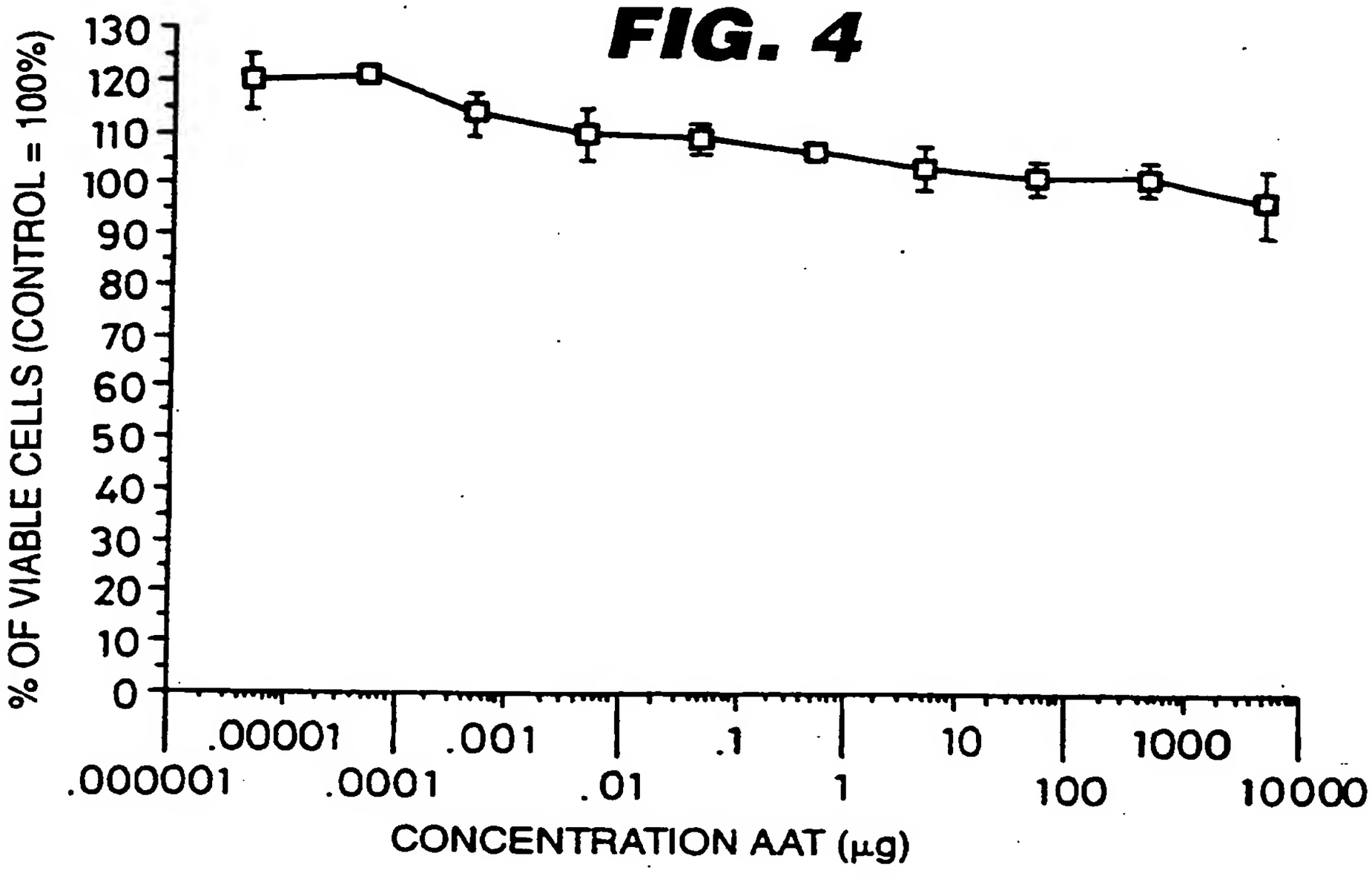

% OF VIABLE CELLS (CONTROL = 100%)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
.00001
.001
.1
10
1000
.000001
.0001
.01
1
100
10000
CONCENTRATION AAT (µg)

**FIG. 4**


% OF VIABLE CELLS (CONTROL = 100%)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
.00001
.001
.1
10
1000
.000001
.0001
.01
1
100
10000
CONCENTRATION AAT (µg)

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# WOUND HEALING COMPOSITIONS CONTAINING ALPHA-1-ANTITRYPSIN

The present invention relates to the use of alpha-1-antitrypsin (AAT) for the preparation of compositions for the treatment of chronic wounds.

alpha-1-antitrypsin (AAT), also known as alpha-1-proteinase inhibitor or Serpin, is a mammalian polypeptide having a molecular weight of approximately 54kDa. It is a potent fluid phase inhibitor of serine proteases, and forms a tightly bound, stoichiomedric complex with elastase. It can be inactivated by cleavage within its reactive centre. For example, neutrophil collagenase (MMP8) is know to degrade and inactivate AAT.

AAT deficiency is a congenital disorder that is principally associated with liver disease in children and emphysema in young adulthood. It is thought that AAT deficiency results in loss of protection in the lung against neutrophil elastase, resulting in breakdown of the architecture of the lung. AAT has been administered in intravenous and aerosol formats for the treatment of pulmonary emphysema.

It is an object of the present invention to provide improved compositions for the treatment of chronic wounds, such as decubitis ulcers, pressure sores and venous ulcers.

The present invention provides the use of alpha-1-antitrypsin for the preparation of a composition for the treatment of a chronic wound.

Preferably, the composition is a wound dressing composition. That to say, the composition is preferably a liquid, semi-solid or solid composition for application directly to the surface of a wound, or the composition is applied to the surface of, or incorporated into, a solid wound contacting layer such as a wound dressing gauze or

film. More preferably, the wound dressing composition is a fluid or a gel comprising from 100ng to 10mg/ml, preferably 10μg to 1mg/ml of AAT in combination with conventional pharmaceutical excipients for topical application to a wound. Suitable carriers include: Hydrogels containing cellulose derivatives, including hydroxyethyl cellulose, hydroxymethyl cellulose, carboxymethyl cellulose, hydroxypropylmethyl cellulose and mixtures thereof; and hydrogels containing polyacrylic acid (Carbopols). Suitable carriers also include creams/ointments used for topical pharmaceutical preparations, e.g. creams based on cetomacrogol emulsifying ointment. The above carriers may include alginate (as a thickener or stimulant), preservatives such as benzyl alcohol, buffers to control pH such as disodium hydrogen phosphate/sodium dihydrogen phosphate, agents to adjust osmolarity such as sodium chloride, and stabilisers such as EDTA.

Alternatively, the wound dressing composition may be a slow release solid composition, in which the AAT is dispersed in a slow release solid matrix such as a matrix of alginate, collagen, or a synthetic bioabsorbable polymer. Preferably, the wound dressing composition is sterile.

Preferably, the chronic wound is selected from the group consisting of venous ulcers, pressure sores, decubitis ulcers, diabetic ulcers and chronic ulcers of unknown etiology. Preferably, the chronic wound is not a periodontal disease condition.

It is to be understood that the term "alpha-1-antitrypsin" as used herein encompasses all naturally occurring polymorphs of AAT. It also encompasses functional fragments of AAT, chimeric proteins comprising AAT or functional fragments thereof, homologs obtained by analogous substitution of one or more amino acids of AAT, and species homologs. Preferably, the AAT is a product of recombinant DNA technology, and more preferably the AAT is a product of

transgenic technology. For example, the gene coding for AAT can be inserted into a mammalian gene encoding a milk whey protein in such a way that the DNA sequence is expressed in the mammary gland, as described in WO88/00239.

Without wishing to be bound by any theory, it is thought that the AAT improves the healing of chronic wounds by inhibiting human neutrophil elastase present in the wound. The healing of such wounds is determined by a complex balance between tissue formation and tissue destruction, and it appears that inhibition of neutrophil elastase by AAT shifts the balance in favour of wound healing.

In another aspect, the present invention provides a method for the treatment of chronic wounds as specified above, the method comprising administering a therapeutically effective amount of AAT to the patient. Preferably, the AAT is administered topically, more preferably in a topical composition as described above.

The present invention now be described further with reference to the accompanying drawings, in which:-

Figure 1 shows the effect of AAT concentration on the activity of human neutrophil elastase in chronic wound fluid as a function of AAT concentration;

Figure 2 shows inhibition of human neutrophil elastase by AAT as a function of AAT concentration;

Figure 3 shows the effect of AAT on the cell viability of rat wound fibroblast (RWF) cells as a function of AAT concentration; and

Figure 4 shows the effective of AAT on the viability of L929 fibroblast cells as a function of AAT concentration.

Procedure 1

AAT is obtained from PPL Therapeutics, East Mains, Ormiston, East Lovian, EH35 5NG, Scotland. The formulation

is produced by transgenic technology by inserting the human gene for the AAT protein into the milk protein gene sequence of sheep. Upon lactation the AAT is purified from the milk and stored as a freeze dried pure protein. Details of the transgenic production method are given in WO88/00239.

AAT from human plasma is also obtainable commercially from Sigma Chemical Company as product A9024.

Procedure 2

The effect of AAT on the activity of human neutrophil elastase (HNE) is studied as follows.

Elastase substrate (31.1mg) supplied by Calbiochem Inc. under catalog reference 324696 is reconstituted in 2ml of pure DMSO in order to solubilise the substrate, and 8ml of buffer (100mM Tris-HCL, pH 7.5, 0.5M NaC1, containing 0.1% Triton®) is added to give a final substrate stock concentration of 5mM. Purified human neutrophil elastase (100$\mu$mg) supplied by Calbiochem Inc. under catalog reference 324681 is reconstituted in 1ml of buffer (100mM Tris-HCL, pH 7.5, 0.5M NaC1, containing 0.1% Triton®).

The neutrophil elastase activity of 50ng of human neutrophil elastase HNE was measured using an elastase substrate concentration of 1mM and determined over an assay period of 1hr at 25°C. The total assay volume is 100$\mu$l. The change in absorbance of the substrate (Meo-Suc-Ala-Pro-Val-pNa) was monitored at 410nm on a 96 well plate. The appropriate substrate blanks are included with each experiment. The ability of the purified neutrophil elastase at 50ng to hydrolyse the substrate (1mM) at varying concentrations was tested in the presence or absence of 20$\mu$ls of AAT (0.5ng-50mg/ml). The percentage inhibition was determined by comparison with a positive control containing elastase and substrate but no AAT, and with a negative control containing substrate but no elastase or AAT.

The results of the study on pure HNE are shown in Figure 2. It can be seen that concentrations of AAT above about 1,000ng/ml substantially inhibit the activity of the HNE.

Likewise, it can be seen from Figure 1 that the activity of HNE present in chronic wound fluid is strongly inhibited by AAT at concentrations above about 2000ng/ml. In Figure 1, the x-axis shows the concentration of AAT in ng/ml, and the y-axis shows the elastase activity of a sample of human wound fluid relative to 50ng/ml of pure HNE.

Procedure 3

The effect of AAT on the viability of cells similar to those found in chronic wounds was assessed as follows.

The basic method is described by Borenfreund and Peurner in Cancer Letters; vol. 34; pages 243-248 (1987), and by Borenfreund *et al.* in Toxicology *in vitro*; vol. 2; pages 1-6 (1988). The method comprises challenging selected cells with the compound to be tested for 3 days, and then determining the number of live cells remaining.

Rat wound fibroblast (RWF) cells of L929 fibroblast cells were removed from tissue culture flasks by trypsination, and counted. The cells were suspended in Dulbecco's Modified Eagle's Medium (DMEM) to give a cell density of $5 \times 10^4$ cells/ml. Aliquots of 100µl, each containing 5000 cells were then dispensed into wells of a 96 well plate.

Samples of AAT were dissolved in DMEM at concentrations of 500pg/ml to 5mg/ml, and 100µl aliquots of these solutions were added to the wells containing the fibroblast cells. Four wells were tested for each AAT concentration. The plates were incubated at 37°C, 5% $CO_2$, humidified atmospheres, for 3 days.

The number of active cells remaining in each well was then determined by the neutral red assay, as described in the references above. Briefly, the assay measures the uptake of the dye neutral red by viable active cells. The dye becomes localized in the lysosomes and is extracted from the cells using an acidic solution. The absorbance of th3e extracted dye is read at 540nm and correlates with the number of active cells.

The results shown in Figures 3 and 4 illustrate that AAT has little or no effect on the viability of the cells tested, even at quite high AAT concentrations. The low cytotoxicity of the AAT indicates that it is suitable for topical application to chronic wounds.

Example 1

An ointment containing AAT and suitable for topical adminstration to a venous ulcer, decubitus ulcer or pressure sore is prepared by mixing the following ingredients in the following percentages by weight:-

| | |
|---|---|
| Freeze-dried AAT | 0.001% |
| Hydroxyethyl Cellulose | 0.35% |
| Carboxymethyl Cellulose | 3.00% |
| Propylene Glycol | 25.00g |
| Sodium Chloride | 0.30% |
| Distilled Water qs to | 100% |

The ointment is entirely wound-friendly and non-cytotoxic, and can be applied to the chronic wound surface at regular intervals until wound healing is achieved.

Many other embodiments of the present invention falling within the scope of the accompanying claims will be apparent to the skilled reader.

SDOCID: <GB 2318732A I >

## CLAIMS

1. Use of alpha-1-antitrypsin for the preparation of a composition for the treatment of a chronic wound.

2. Use according to claim 1, wherein the composition is a wound dressing composition.

3. Use according to claim 2, wherein the wound dressing composition is a fluid or a gel comprising from 10 to 1000μg/ml of alpha-1-antitrypsin in combination with conventional pharmaceutical excipients for topical application to a wound.

4. Use according to claim 1, 2 or 3, wherein the chronic wound is selected from venous ulcers, pressure sores, decubitis ulcers and diabetic ulcers.

5. Use according to any preceding claim, wherein the alpha-1-antitrypsin is a product of transgenic technology.

The Patent Office

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**Application No:** GB 9622805.1
**Claims searched:** 1-5

**Examiner:** Dr Carol Davies
**Date of search:** 19 February 1997

# Patents Act 1977
# Search Report under Section 17

**Databases searched:**

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): A5B (BHA, BJB)

Int Cl (Ed.6): A61K 38/55, 38/57; C07K 14/81

Other: ONLINE: CAS-ONLINE; PHARM; WPI

**Documents considered to be relevant:**

| Category | Identity of document and relevant passage | Relevant to claims |
|---|---|---|
| X | EP 0648838 A1 (AMGEN INC.) see p.9 lines 30-48 in particular line 46; p.11 lines 47-57; claims 48, 49 & 52. | 1 at least |
| X | J. Invest. Dermatol., (1995), 105 (4), pages 572-578. See the second column on p.572; column 1 on p. 575 paragraph entitled "degradation of FN by chronic wound fluid proteinases was inhibited by AT"; the discussion on p.576-7 especially the last paragraph on p.577. | 1 at least |

| | | | |
|---|---|---|---|
| X | Document indicating lack of novelty or inventive step | A | Document indicating technological background and/or state of the art. |
| Y | Document indicating lack of inventive step if combined with one or more other documents of same category. | P | Document published on or after the declared priority date but before the filing date of this invention. |
| & | Member of the same patent family | E | Patent document published on or after, but with priority date earlier than, the filing date of this application. |

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